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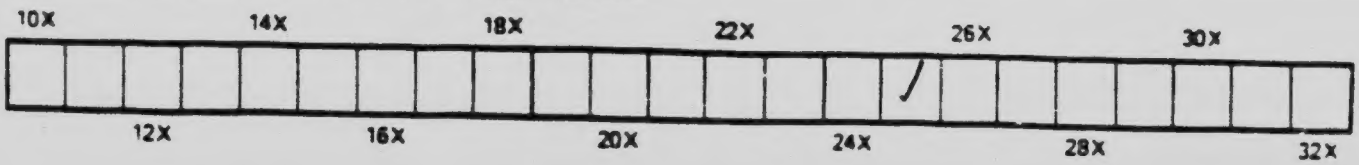

UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 10: ON THE PROBABLE NATURE OF THE SUBSTANCE PROMOTING GROWTH IN YOUNG ANIMALS, BY CASIMIR FUNK AND ARCHIBALD BRUCE MACALLUM

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STUDIES ON GROWTH.

II. ON THE PROBABLE NATURE OF THE SUBSTANCE PROMOTING GROWTH IN YOUNG ANIMALS.

BY CASIMIR FUNK AND ARCHIBALD BRUCE MACALLUM.[1]

(From the Cancer Hospital Research Institute, London, and the Biochemical Laboratory, University of Toronto.)

(Received for publication, October 8, 1915.)

Since the remarkable work of Osborne and Mendel,[2] Hopkins,[3] and McCollum and Davis,[4] who have shown that a young animal requires something special besides the usual food constituents for its process of growth, there has been much discussion as to the exact nature of this product. In our first paper[5] we discussed this subject and pointed out that most workers regard the growth factor as being closely associated with fats. The experimental evidence which led to this opinion was brought forward first by McCollum, but has become especially significant since Osborne and Mendel[6] and later Osborne and Wakeman[7] found that purified butter, which in their opinion was free from nitrogen, was still able to promote growth in young rats. In our earlier paper we found that even the purified butter contains traces of nitrogenous substances, and therefore might possibly contain traces

[1] The work was begun at the Cancer Hospital Research Institute, London, England; the experiments illustrated by the curves and tables were carried out in the Biochemical Laboratory, University of Toronto, during the tenure of a Senior Research Fellowship in the Department of Medical Research, and the expenses were defrayed by a grant from this department.

[2] Osborne, T. B., and Mendel, L. B., *Carnegie Institution of Washington, Publication No. 156*, pts. i and ii, 1911.

[3] Hopkins, F. G., *Jour. Physiol.*, 1912, xliv, 425.

[4] McCollum, E. V., and Davis, M., *Jour. Biol. Chem.*, 1913, xv, 167.

[5] Funk, C., and Macallum, A. B., *Ztschr f. physiol. Chem.*, 1914, xcii, 13.

[6] Osborne and Mendel, *Jour. Biol. Chem.*, 1913–14, xvi, 423.

[7] Osborne, T. B., and Wakeman, A. J., *Jour. Biol. Chem.*, 1915, xxi, 91.

of vitamines, an opinion which is shared by McCollum and Davis.[8] Recently MacArthur and Luckett[9] have found that the growth-promoting substance is not contained in the ether-soluble fraction, and they also suggest the possibility of vitamines as a factor.

To complete our first paper we carried out a series of experiments on young rats with ordinary butter and purified butter fat as the fat fraction of the diet. Both with butter and purified butter fat all the animals died after five to seven weeks, although on the former diet a slight initial advantage was noticed. Repeating these experiments on pigeons we convinced ourselves of the inability of both diets to prevent the onset of beri-beri symptoms, indicating the absence of or an insufficient quantity of beri-beri vitamine.

In our subsequent experiments we increased the percentage of butter and pure butter fat in the diets from 12 to 30 per cent with the same negative result in all cases. On both diets the rats showed, twenty-four to forty-eight hours before death, a condition of spastic contraction resembling somewhat avian beri-beri or infantile tetany.

We also carried out experiments in which increasing amounts of starch were replaced by equivalent quantities of unpolished rice, with lard as the fat fraction of the diet. The results, which were tending to support the vitamine theory of growth of one of us,[10] were still unsatisfactory, although the diet containing the largest percentage of unpolished rice proved to be much better both for maintenance and growth than those diets in which butter was used; yet this was finally inadequate as the animals declined after nine weeks.

Finally we made up diets, with butter used as fat, to which from 2 to 6 per cent of dried brewer's yeast was added. On this diet we have obtained successful growth and maintenance. As the growth-promoting factor is beyond question contained in yeast, we intend to fractionate the yeast and show which fraction contains the hypothetical growth substance.

The butter was purified as indicated in our earlier paper.[5] The casein was purified by extraction with hot alcohol. The fuel

[8] McCollum and Davis, *Jour. Biol. Chem.*, 1914, xix, 245.
[9] MacArthur, C. G., and Luckett, C. L., *Jour. Biol. Chem.*, 1915, xx, 161.
[10] Funk, C., *Ztschr. f. physiol. Chem.*, 1913, lxxxviii, 352.

value of the food and also the amount of the food absorbed from the intestine were controlled by means of an adiabatic calorimeter. The food mixture used, with the exception of the butter, was found to produce beri-beri in earlier experiments by one of us[11] on pigeons. Special experiments were carried out in order to ascertain the value of a food mixture consisting of casein, starch, cane-sugar, salt mixture, and butter for pigeons. All the pigeons fed on this food developed a typical beri-beri and no difference was noticed between the ordinary butter and the purified butter.

The results presented in this paper are a selection of a large number of experiments of uniform character. Two rats of the same sex were used in each experiment. The four diets used had the following composition.

Diet.	I.	II.	III.	IV.
	per cent	per cent	per cent	per cent
Casein	22	22	22	22
Sugar	10	10	10	10
Starch	33	33	31	27
Butter (ordinary)	30		30	30
(purified)		30		
Agar	2	2	2	2
Salt mixture*	3	3	3	3
Yeast (dry)			2	6

* The composition of the salt mixture was the same as in the experiments of Osborne and Mendel.[12]

Experiment I.—Chart I. The curves represent the average weight of two rats each of which was fed on Diet I (ordinary butter). As represented by the upper curve the rats showed a slight initial gain in weight and maintenance for about 20 days; then a rapid decline set in with fatal termination after 36 days. The lower curve represents the average weight of two rats which were changed from Diet I to Diet IV on the twentieth day. The effect of the addition of dried brewer's yeast was striking; the rats suddenly recovered and grew normally up to the end of the experiment. The intake of food and the absorption from the intestine are recorded below.

[11] Funk, *Ztschr. f. physiol. Chem.*, 1914, lxxxix, 373.
[12] Osborne and Mendel, *Jour. Biol. Chem.*, 1913, xv, 311.

	Upper curve, Rats 25 and 26.				Lower curve, Rats 27 and 28.			
Days.	Average weight.	Food intake.	Food.	Feces.	Average weight.	Food intake.	Food.	Feces.
	gm.	gm.	cal.	cal.	gm.	gm.	cal.	cal.
0	26.5				21.0			
4	32.25	25.1	120.5	3.67	27.5	18.15	89.2	3.09
8	36.5	19.6	95.4		26.5	15.0	74.7	
12	37.5	21.1	103.7	3.37	27.5	18.65	91.7	2.29
16	37.0	21.3	103.6		26.5	17.5	85.5	
20	33.0	25.0	125.1	2.66	27.0	25.65	134.2	5.42
24	30.5	21.5	111.2		38.0	43.2	229.5	
28	28.5	14.5	74.5	4.36	40.5	42.1	223.4	9.75
32	26.5	10.4	53.2		62.5	53.66	285.8	
36	23.5	10.2	52.2	2.23	73.5	57.95	308.8	16.60
40	Died				81.5	53.8	286.5	
44					85.0	54.15	288.5	
48					92.0	58.0	309.5	

This experiment was repeated on six male and two female rats with identical results.

Experiment II.—Chart II. Here we have used purified butter fat. Each curve represents the average weight of two male rats. The results are similar to those of the first experiment, only the maintenance period was slightly shortened. The same marked recovery was observed on changing from Diet II to Diet IV. The details of the experiment are recorded below.

	Upper curve, Rats 31 and 32.				Lower curve, Rats 29 and 30.			
Days.	Average weight.	Food intake.	Food.	Feces.	Average weight.	Food intake.	Food.	Feces.
	gm.	gm.	cal.	cal.	gm.	gm.	cal.	cal.
0	43.5				29.5			
4	49.0	32.2	176.8	8.34	36.5	25.85	141.2	4.25
8	52.0	26.35	143.9		39.5	18.3	99.9	
12	48.5	23.4	127.8	7.35	42.0	17.8	97.2	3.58
16	46.0	23.8	129.8		39.0	18.65	101.8	
20	44.5	23.75	129.6	8.00	36.0	17.75	96.9	3.43
24	42.0	24.1	131.5		31.5	17.65	96.6	
28	42.5	18.5	109.0	3.95	48.0	43.25	229.5	8.04
32	39.5	14.15	77.2		58.5	54.45	290.2	
36	36.5	11.85	68.2		74.5	61.85	324.3	13.09
40	32.5	6.70	36.79		86.0	53.5	285.0	
44	Died				94.5	57.1	304.2	
48					103.0	61.70	328.8	

This experiment was repeated on seven male rats and one female with the same result.

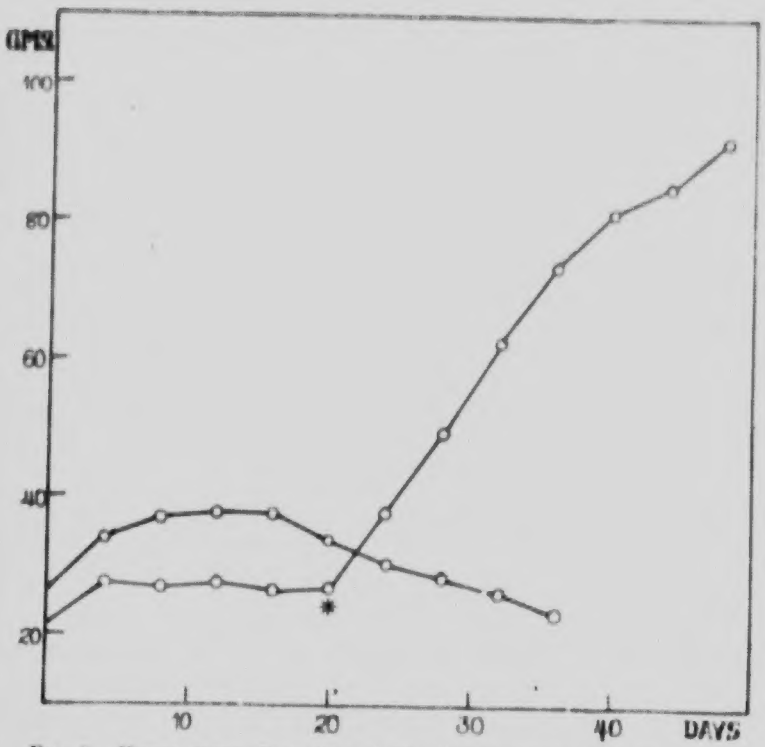

FIG. 1. Upper curve, Rats 25 and 26. Lower curve, Rats 27 and 28. On the curve at the point (*) Diet I was changed to Diet IV; Rats 25 and 26 died after 36 days on Diet I.

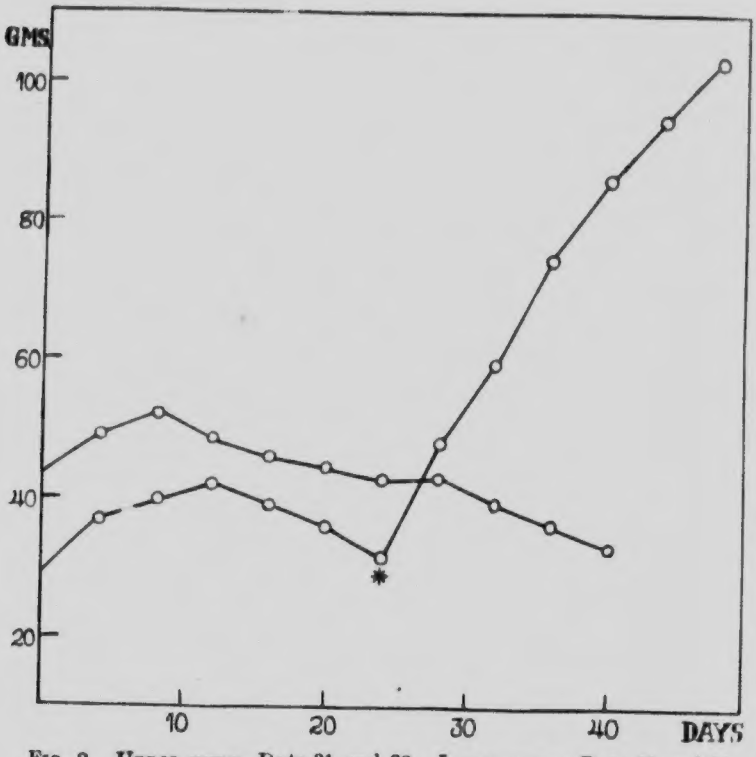

FIG. 2. Upper curve, Rats 31 and 32. Lower curve, Rats 29 and 30. At the point (*) on lower curve Diet II was changed to Diet IV. Rats 31 and 32 died after 40 days on Diet II.

Experiment III.—Chart III. In this experiment the upper curve illustrates the weight of two male rats, and the lower curve the weight of two female rats. Up to 20 days Diet III was used (with 2 per cent yeast), being then replaced by Diet IV. In one set this was continued to the end of the experiment (lower curve). In the other experiment Diet IV was replaced after 32 days, by Diet II (without yeast); the growth ceased abruptly and the animals rapidly declined. No marked difference was noticed between the diets containing 2 and 6 per cent of yeast. The details of the experiment are recorded below.

	Upper curve, Rats 33 and 34.				Lower curve, Rats 35 and 36.			
Days	Average weight.	Food intake.	Food.	Feces.	Average weight.	Food intake.	Food.	Feces.
	gm.	*gm.*	*cal.*	*cal.*	*gm.*	*gm.*	*cal.*	*cal.*
0	20.0				18.5			
4	29.0	29.1	151.2	5.31	27.5	26.2	136.2	3.41
8	35.0	23.05	117.3		32.0	19.4	97.9	
12	37.0	25.95	132.4	7.30	37.0	26.65	137.1	4.39
16	41.5	26.60	137.2		39.5	29.85	148.1	
20	43.5	30.85	161.5	6.45	42.5	34.15	178.7	7.44
24	47.0	41.15	217.5		49.5	44.6	236.8	
28	50.0	44.0	233.5	8.06	58.0	43.5	230.9	11.13
32	60.0	49.5	262.6		69.5	48.75	259.5	
36	72.5	41.05	225.5	9.53	80.0	60.70	323.5	13.68
40	74.5	34.15	187.7		87.5	56.70	302.2	
44	71.0	30.85	169.6		90.0	51.90	276.5	
48	68.5	32.90	180.9		94.0	58.25	310.4	

This experiment was repeated with two additional female rats with the same result.

Our new series of experiments clearly show the absolute inability of either butter or purified butter fat to stimulate the growth of young rats. This result could only be expected from our experiments with the same diet on pigeons, which have shown the absence of vitamine in butter. From the tables we see a strict relationship between the growth observed and the food taken in. We also notice that the addition of yeast stimulates, directly or indirectly, the appetite. The calorific determination of the food and feces shows in all cases a practically complete absorption of the food by the digestive tract, as the calorific value of the feces represents but from 2.5 to 5 per cent of the calories taken in by

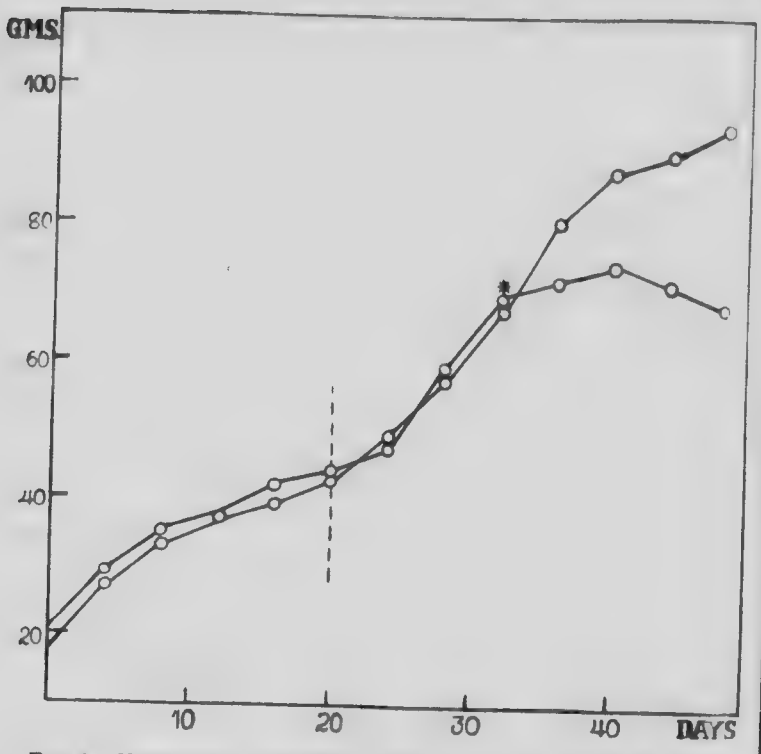

FIG. 3. Upper curve, Rats 33 and 34. Lower curve, Rats 35 and 36. To the left of the dotted line Diet III; to the right, Diet IV. At the point (*) Rats 33 and 34 were changed from Diet IV to Diet II.

the animals. Whether yeast alone without butter (replaced by lard) will produce normal growth in rats, and whether there are one or more components in yeast which stimulate growth, is under investigation.

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